probing cooperative interactions between distant DNA regulatory sites that would have been difficult to test otherwise.

3058-Plat
The Primary DNA-Binding Subsite of the Rat Pol Beta. Energetics of Interactions of the 8-kDa Domain of the Enzyme with the ssDNA
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Interactions of the 8-kDa domain of the rat pol β and the intact enzyme with the ssDNA have been studied, using the quantitative fluorescence titration technique. The 8-kDa domain induces large topological changes in the bound DNA structure and engages much larger fragments of the DNA than when embedded in the intact enzyme. The DNA affinity of the domain is predominantly driven by entropy changes, dominated by the water release from the protein. The thermodynamic characteristics dramatically change when the domain is embedded in the intact polymerase, indicating the presence of significant communication between the 8-kDa domain and the catalytic 31-kDa domain. The diminished water release from the 31-kDa domain strongly contributes to its dramatically lower DNA affinity, as compared to the 8-kDa domain. Unlike the 8-kDa domain, the DNA binding of the intact pol β is driven by entropy changes, originating from the structural changes of the formed complexes.

3059-Plat
Turning on the Spliceosome
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The spliceosome is the complex macromolecular machine responsible for removing introns from pre-mRNAs. The processes of spliceosome assembly and activation rely on the coordinated interactions of many dozens of spliceosome components to identify splice sites in a pre-mRNA, build a spliceosome, and activate the spliceosome for catalysis by formation of an active site prior to transesterification. The activation step itself likely involves many intermediates. It results in loss of the U1 and U4 snRNPs from the spliceosome, removal of SF3 from the branchsite, and several conformational rearrangements of the snRNAs and pre-mRNA prior to lariat formation. These assembly and activation events are best studied using endogenous spliceosome components found in whole or nuclear cell extracts. We recently demonstrated that a single molecule technique (CoSMoS: Co-localization Single Molecule Spectroscopy) in combination with yeast genetic engineering and chemical biology provides a powerful method for studying spliceosome assembly in S. cerevisiae whole cell lysate (Hoskins et al., Science, v331, pg. 1289-95 (2011)). That study provided significant novel insight into the kinetics of the assembly reaction. We are now extending these results to spliceosome activation. By monitoring the relative association and dissociation kinetics of the U1, U4, U5, NTC, and SF3b spliceosome components on single pre-mRNAs, we are able to definitively order the snRNP association and dissociation events involved in spliceosome activation.

3060-Plat
Deletion of Ribosomal Cofactor Rimp Disrupts Late Stage 30S Subunit Assembly
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The bacterial 70S ribosome, consisting of the 30S and 50S subunits, facilitates protein synthesis and is a target for antibiotics. During 30S biogenesis, 16S ribosomal ribonucleic acid (rRNA) is co-transcriptionally processed by ribonucleases, and bound by ribosomal proteins (RPs) and cofactors. However, the coordination between rRNA processing, and cofactor and RP binding is unclear. Here, we reveal that deletion of the ribosomal cofactor gene, *rimP*, disrupts binding of specific RPs (S2, S12, S21) during the late stages of 30S assembly in *Escherichia coli*. We use a stable isotope labeling/mass spectrometry approach to show that the *rimP* deletion strain accumulates 30S assembly intermediates lacking the late assembly binders, S2, S12 and S21, with a marked delay in 30S assembly relative to 50S assembly. Further studies will determine the extent of rRNA processing in 30S assembly intermediates in the *rimP* deletion strain, towards elucidating the coordination between rRNA processing, RP binding and cofactor function.

3061-Plat
Real Time Monitoring of DNA Bending and Unbending by E. Coli Integration Host Factor
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Integration Host Factor (IHF) is an abundant nucleoid associated protein in E.Coli that binds to and contributes to organizing the chromosomal DNA by non-specific protein-DNA interaction. In addition to the role in chromosomal DNA organization, IHF is also an indispensable part of site-specific integration of bacteriophage lambda into the host genome. This function of IHF largely originates from its ability to bind DNA with high specificity and bend the DNA about 160° at its binding sites. Using a novel magnetic tweezers instrument that allows manipulation of very short DNA tethers, we studied the interaction of one IHF with a specific binding sequence (H' sequence) inserted into the middle of a 534-bp DNA fragment. Fluctuation between two distinct DNA extensions is observed in real-time at < 1 pN forces, which corresponds to the bending conformation and unbending conformation of an IHF/DNA complex (shown in figure below). Effects of environmental factors, such as osmolality, temperature, and IHF concentration are investigated in details. Our results have shed a light on understanding the properties of the IHF-H' complex, which may improve our understanding of its role in the λ-integration process.

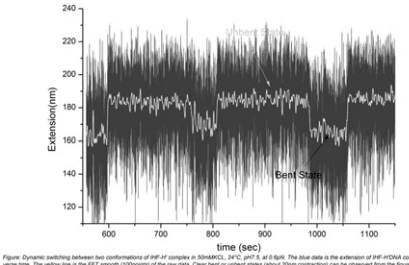

Figure: Dynamic switching between two conformations of IHF-H' complex in 50mMKCl, 24°C, pH7.5, at 0.6pN. The blue data is the extension of IHF-H'DNA complex extension verse time. The yellow line is the FFT smooth (100points) of the raw data. Clear bent or unbent states (about 20nm contraction) can be observed from the figure.

Platform: Voltage-gated Na Channels

3062-Plat
Calcium Dysregulation of Voltage-Gated Sodium Channels Harboring LQT3 Mutations
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Intracellular calcium ions modulate sodium channel inactivation by producing a depolarizing shift the steady-state inactivation equilibrium. We have recently proposed a mechanism for this effect by which direct Ca2+/calmodulin(CaM) binding to the inactivation gate increases the transient availability of channels in the action potential by shifting the steady-state inactivation. Interestingly, a crystal structure of Ca2+/CaM bound to the inactivation gate of the sodium channel pinpoints the position of four mutations (M1498T, K1500Δ, L1501V, G1502S) shown previously to underlie long QT3 syndrome, along a critical binding interface. We explored the possibility that these mutations, in addition to effects on channel gating, may alter calcium regulation of Nav1.5. This possibility was first tested directly with Isothermal Titration Calorimetry (ITC) to determine the binding parameters of purified proteins and then by patch-clamp electrophysiology of expressed wild-type and mutant channels. Interestingly, ITC experiments demonstrated that the mutations impacted Ca2+/CaM binding by altering the affinity of Ca2+/CaM for the inactivation gate. Channels carrying inherited mutation showed robust expression in HEK-293 cells with either modest or severe effects on channel gating, as expected for LQT3 mutations. However, in terms of Ca2+ regulation, the LQT3 mutations significantly altered the calcium-induced shift in steady-state inactivation compared to wild-type channels. The data suggest that calcium dysregulation of the voltage-gated sodium channel may contribute to the pathogenesis of LQT3 syndrome.

3063-Plat
Single Channel Studies and Kinetic Modeling of an Inactivation Deficient Voltage-Gated Sodium Channel
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Rapid inactivation is a hallmark of voltage-gated sodium channels critical for regulating the rate of electrical signaling between excitable cells. However, activation and inactivation processes can overlap making it difficult to determine which process is altered during a given perturbation. Moreover, at the single channel level rapid entry into inactivated states occludes less frequent channel activity. Thus, removing inactivation should simplify the interpretation of macroscopic effects and reveal the intrinsic gating behavior associated with channel